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The pattern recognition molecule ficolin-1 exhibits differential binding to lymphocyte subsets, providing a novel link between innate and adaptive immunity

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ABSTRACT

Ficolin-1 is a soluble pattern recognition molecule synthesized by myeloid cells and capable of activating the lectin pathway of complement on the surface of pathogens. It is tethered to the membranes of monocytes and granulocytes; however, the biological significance of cell-associated ficolin-1 is unknown. Recognition of healthy host cells by a pattern recognition molecule constitutes a potential hazard to self cells and tissues, emphasizing the importance of further elucidating the reported self-recognition. In the current study we investigated the potential recognition of lymphocytes by ficolin-1 and demonstrated that $CD56^{dim}$ NK-cells and both $CD4^+$ and $CD8^+$ subsets of activated T-cells were recognized by ficolin-1. In contrast we did not detect binding of ficolin-1 to $CD56^{bright}$ NK-cells, NKT-cells, resting T-cells or B-cells. Furthermore, we showed that the protein–lymphocyte interaction occurred via the pathogen-recognition domain of ficolin-1 to sialic acid on the cell surface. Thus, the differential binding of ficolin-1 to lymphocyte subsets suggests ficolin-1 as a novel link between innate and adaptive immunity. Our results provide new insight about the recognition properties of ficolin-1 and point toward additional immune modulating functions of the molecule besides its role in pathogen recognition.

1. Introduction

The immune system is traditionally divided into the two arms of innate and adaptive immunity that collaborate to eliminate pathogens and endogenous debris from the organism. Innate immunity exerts a variety of induced effector mechanisms to contain early infection until the adaptive immune response takes effect. This first-line of host defense carried out by the innate immune system relies on evolutionary conserved pattern recognition molecules (PRMs) that recognize structures present on invading pathogens and altered host cells (Medzhitov and Janeway, 2000; Medzhitov, 2009). Among these PRMs are the ficolins, a family of proteins present in species ranging from invertebrates to mammals (Matsushita, 2010). Ficolins are initiators of the lectin pathway of complement; hence their recognition of carbohydrates on the surface of pathogens or altered host-cells promotes the activation of the mannose binding lectin (MBL)/ficolin-associated serine proteases (MASPs) which lead to subsequent cleavage and activation of other complement factors (Matsushita, 2013).

Three human ficolin genes have been identified: *FCN1*, *FCN2* and *FCN3*, encoding ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin), respectively. The *FCN*s encode a polypeptide consisting of an N-terminal region followed by a collagen-like domain and a C-terminal fibrinogen-like domain (FBG-domain). Three identical polypeptides are assembled into trimeric structural subunits and further folded into higher oligomeric forms. The collagen-like domain interacts with the MASPs and the FBG-like domain is involved in ligand recognition. Ficolin-1 and ficolin-2 are 79% identical at the amino acid level, whereas ficolin-3 is 48% homologous with ficolin-1 and ficolin-2 (Garred et al., 2010; Hummelshoj et al., 2007; Sugimoto et al., 1998; Ohashi and Erickson, 1998).

The ficolins exhibit differences in tissue expression and ligand specificity, suggesting individual roles of each ficolin. Ficolin-2 mRNA is expressed in the liver (Matsushita et al., 1996) while ficolin-3 mRNA is expressed in the liver and lung (Sugimoto et al., 1998; Akaiwa et al., 1999; Hummelshoj et al., 2008). Ficolin-1 mRNA is predominantly expressed in bone marrow and in peripheral blood cells of myeloid lineage (Hummelshoj et al., 2008; Liu

Abbreviations: FBG-domain, fibrinogen-like domain; FCS, fetal calf serum; FITC, flourescein isothiocyanate; GlcNac, N-acetyl-D-glucosamine; mAb, monoclonal antibody; MASP, mannose-binding lectin/ficolin-associated serine protease; MBL, mannose-binding lectin; PBMCs, peripheral blood mononuclear cells; PRM, pattern recognition molecule; PWM, pokeweed mitogen; rFicolin-1, recombinant ficolin-1; SNA, samubucus nigra.

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et al., 2005; Lu et al., 1996). All three ficolins are present in serum with ficolin-1 being the least abundant (Munthe-Fog et al., 2007, 2008, 2012; Honore et al., 2008; Wittenborn et al., 2010). Ficolin-1 is localized in cytoplasmatic granules of monocytes and of neutrophils, from where it is secreted into the surroundings (Liu et al., 2005; Honore et al., 2008; Rorvig et al., 2009). In addition ficolin-1 is found as a membrane-associated protein on the surface of these cells (Honore et al., 2010).

Although the ficolins share a common affinity toward acetylated compounds, there is a great variation in the binding specificity of the individual ficolins (Matsushita, 2013). A peculiar property of ficolin-1 is its ability to bind to sialic acid, a feature of normal viable cells (Gout et al., 2010). In addition, ficolin-1 binds to other acetylated carbohydrates such as N-acetyl-D-glucosamine (GlcNAc) and N-acetyl-D-galactosamine (GalNAc) and to the bacteria *Staphylococcus aureus* (Liu et al., 2005). A recent study has shown that ficolin-1 binds to capsulated isolates of Group B Streptococcus via sialic acid mediating complement activation (Kjaer et al., 2011). Ficolin-2 binds to a wide range of pathogens including bacteria, fungi and viruses, whereas only a few bacterial ligands have been identified for ficolin-3 (Matsushita, 2013).

Moreover, the ficolins are involved in crosstalk with soluble pentraxins such as C-reactive protein (CRP) and pentraxin 3 (PTX3) (Ng et al., 2007; Tanio et al., 2009; Ma et al., 2009; Gout et al., 2011). More specifically, ficolin-1 binds to CRP enhancing the binding of CRP to bacteria (Ng et al., 2007). Another study has shown an inhibitory effect of CRP on ficolin-1 mediated secretion of interleukin-8 from monocytes (Zhang et al., 2010). Similarly, the ficolin-1–PTX3 interaction on apoptotic cells has recently been shown to enhance phagocytosis by macrophages and down regulate interleukin-8 production in the macrophages during phagocytosis, thereby acting as a non-inflammatory eat-me signal to sequester dying host cells (Ma et al., 2013).

Ficolin-1 is tethered to the surface of monocytes and neutrophils despite being devoid of a transmembrane segment, and it has been reported that the membrane binding occurs via binding of the FBG-domain of ficolin-1 to sialic acid on the cell surface (Rorvig et al., 2009; Honore et al., 2010; Teh et al., 2000). However, it has also been suggested that the FBG-domain of ficolin-1 is associated to G protein-coupled receptor 43 on the surface of monocytes (Zhang et al., 2010). In another recent study it was reported that ficolin-1 interacts with the main membrane sialoprotein of neutrophils, CD43 (Moreno-Amaral et al., 2012). It is possible that ficolin-1 functions as a secretory molecule, that binds to the surface of ficolin-1 producing cells, and thereby modulates cellular functions in an autocrine manner, but the biological role of ficolin-1 binding to healthy monocytes and neutrophils remains to be elucidated.

Interestingly, we have previously shown that the activation of T-cells induced an up regulation of surface-expressed sialic acid accompanied by a profound binding of ficolin-1 to the activated T-cells (Honore et al., 2010). Recognition of healthy host cells by a pattern recognition molecule constitutes a risk of harmful complement activation on the surface of these cells, and this emphasizes the importance of elucidating the reported self-recognition. Thus, the objectives of the current study were to investigate the potential recognition of lymphocyte subsets by ficolin-1and to identify the putative ligands on the surface of these cells.

2. Materials and methods

2.1. Materials

RPMI-1640 medium, penicillin/streptomycin solution, L-glutamine, GlcNAc, D(+)-mannose, sialic acid and sialidase from *Clostridium perfringens* were purchased from Sigma–Aldrich (Brøndby, Denmark). $CaCl_2$, NaCl and $MgCl_2$ were obtained from Merck (Darmstadt, Germany). Fetal calf serum (FCS) was purchased from Invitrogen (Taastrup, Denmark). Phosphate-buffered saline (PBS) (10 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and Tris-buffered saline (TBS) (10 mM Tris, 150 mM NaCl, pH 7.4) were obtained from Bie & Berntsen (Rødovre, Denmark). FITC-conjugated streptavidin was purchased from DAKO (Glostrup, Denmark). A mouse monoclonal antibody (mAb) against human ficolin-1 (clone 106) was generated and characterized in our laboratory (Honore et al., 2008) and biotinylated using an immunoprobe biotinylation kit, according to the manufacturer's instructions (Sigma–Aldrich). Biotin Mouse IgG isotype control, as well as cell surface markers CD3-PerCP, CD56-APC, CD25-PE, CD4-APC, CD8-APC, CD19-PE were obtained from BD Biosciences (San Jose, CA, USA). FITC-conjugated *Samubucus nigra* bark lectin (SNA-FITC) was obtained from Vector Laboratories (Burlingame, CA, USA). Lymphoprep was purchased from Axis-Shield (Oslo, Norway).

2.2. Recombinant ficolin-1

Recombinant ficolin-1 (rFicolin-1) was generated as described previously for ficolin-2 (Hummelshoj et al., 2007). Briefly, the FCN1 gene was amplified and tagged with a hexa-His sequence in the C-terminal end. The constructs were cloned into an expression vector and transfected into the Chinese hamster ovarian (CHO) cell line DG44. His-tagged rFicolin-1 was purified from the CHO cell supernatant on a nickel-nitrilotriacetic acid column (Qiagen).

2.3. Isolation of peripheral blood mononuclear cells (PBMCs)

Venous blood from healthy donors was drawn in heparinized Vacuette collection tubes (Greiner Bio-One, Frickenhausen, Germany), diluted 1:1 in PBS, layered on top of a Lymphoprep gradient and centrifuged according to the manufacturer's instructions.

2.4. Lymphocyte stimulation

PBMCs were washed twice in RPMI containing penicillin/streptomycin, L-glutamine and 10% heat-inactivated fetal calf serum (FCS). Cells (0.5×10^6) were seeded into six-well plates (Techno Plastic Products, Trasadingen, Switzerland) in media as above and incubated with pokeweed mitogen (PWM) (Sigma–Aldrich) at a concentration of 0.33 μg/ml for 4 days at 37 °C in 5% CO_2 and 95% humidity. Successful stimulation of T-cells was analyzed by flow cytometry using fluorescence-conjugated anti-CD25 (BD Biosciences).

2.5. Ficolin-1 binding to cells

Cells (2×10^5 cells/ml) were washed and resuspended in TBS-buffer containing 5 mM $CaCl_2$, 1 mM $MgCl_2$ and 1% heat-inactivated FCS, pH 7.4 (TBS/HI-FCS), followed by incubation with rFicolin-1 for 1 h at 37 °C. In inhibition experiments TBS/HI-FCS also contained GlcNAc, D(+)-mannose or sialic acid. For calcium-dependent studies, 5 mM $CaCl_2$ was replaced with 10 mM EDTA or 10 mM EGTA + 5 mM $MgCl_2$ in the TBS-buffer (pH adjusted to 7.4). All reaction volumes were 100 μl and cells were washed after each step in TBS/HI-FCS. Bound ficolin-1 was detected using 10 μg/ml biotinylated FCN106 (30 min at 4 °C) followed by fluorescence conjugated streptavidin (30 min at 4 °C). Finally, cells were stained with various surface markers (15 min at 4 °C) washed and resuspended in 200 μl cold TBS/HI-FCS. Relevant controls were included routinely in the experiments. These controls included negative